THE EFFECT OF OVARIECTOMY AND OF THE INJECTION OF EXTRACTS OF THE CORPUS LUTEUM ON PREGNANCY IN RATS

by

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INTRODUCTION

Theories regarding the functions of the corpus luteum have been prevalent since its existence in the ovary was first known. A large amount of experimental work has been done in an effort to definitely determine its activities and to elucidate the degree to which it is essential for the maintenance of pregnancy. That the corpus luteum is necessary for the attachment and development of the early embryo has been definitely established. Authors are, however, not agreed as to how long it is subsequently required or as to exactly what other functions may be attributed to it.

REVIEW OF LITERATURE

Early views were that the corpus luteum prevented hemorrhages and the formation of scar tissue, or preserved the continuity of the ovarian circulation. It was held by de Graaf (1672) according to Asdell (1928) to be formed only after copulation.

Probably the first suggestion of the corpus luteum as a gland of internal secretion and likewise the first to withstand criticism was that made by Beard (1897), namely, that it was responsible for the suppression of ovulation during gnancy. Born according to Corner (1923) in 1900 advanced ew that the corpus luteum prepared the uterus for the reception and imbedding of the fertilized ovum by means of an internal secretion. The general belief at this time was that the decidual changes in the uterus during pregnancy were brought about by a stimulus from the embryo. Born observing that there was a close correlation in the existence of the corpus luteum with pregnancy and also that decidual changes occurred in the uterus in tubal pregnancy, suggested the dependence of the decidual preparation of the uterus upon the corpus luteum. It was through his influence that Fraenkel, a pupil of Born, was persuaded to put this hypothesis to experimental tests.

Fraenkel (1903) chose the rabbit for his experiments. He attacked the problem by removing the ovaries from a series of ten animals during the first seven days after coitus, before implantation had occurred, and by entirely ablating the corpora lutea in 48 animals. Another series of 70 animals in the various stages of pregnancy up to fourteen and eighteen days was treated in the same manner. In all of these cases the pregnancies were interrupted. In his control animals where one ovary or one or more corpora lutea were not removed the pregnancy was continued to full term. He found that the presence of one corpus was sufficient to maintain the pregnancy. Extirpation of the corpora lutea after the twentieth day was sometimes without effect. He demonstrated clearly in these experiments that the corpus luteum is essential for the attachment of the fertilized ovum to the uterine mucosa and also for the subsequent maintenance of foetal nutrition. He failed however to find the corpus luteum essential in the later stages of pregnancy and concluded as a result that it was required only during the early stages after implantation.

This classic work by Fraenkel served as an impetus for further work. His results were soon confirmed by other workers for the rabbit and also for other animals. Marshall and Jolly (1905) reported similar results from a series of experiments for which they had chosen the dog and rat. Ovaries were removed from dogs at stages from three days to four weeks after copulation, with the result that the pregnancies did not continue. Ovaries were removed from ten rats that had been with males for several days and no pregnancies followed. Ovaries were removed from nine rats in early stages and no pregnancies continued,but when ovaries were removed from a rat at 13 days and also from two rats at 21 days of pregnancy, full term followed. The removal of only one ovary had no effect on the pregnancy independent of the stage. They concluded from their work that the corpus luteum produces a secretion essential for the attachment and development of the early embryo but that it is not functional in the later stages of pregnancy.

Fraenkel's results were further confirmed by Ancel and

Bouin (1910) for the rabbit and by Kleinhaus and Shenk (1908) for the guinea pig and rabbit. Clinical cases on record by Allen, Pratt, and Doisy (1925) and others suggest that in the human the corpus luteum of pregnancy or even the entire ovaries may be removed quite early with no adverse results. More recent work seems, however, to indicate that in most animals the corpus luteum is essential throughout the pregnancy. Weymeersch (1907) and Hammond (1927) have reported that double ovariectomy during pregnancy in the rabbit is invariably followed by resorption or abortion. Drummond, Robinson and Asdell (1926) came to the same conclusion for the goat. Hartman (1925) for the opossum, Dripps (1919) for the spermophile, Harris (1927) and Parkes (1928 a) for the mouse found the corpora lutea essential throughout the pregnancy. Hess (1921), Wester (1921) and Schmaltz (1921) according to Parkes (1929) report the same for the cow. Herrick (1928), working in this laboratory, concluded that in the guinea pig extirpation of both ovaries usually causes abortion but that in some instances the pregnancy may ccontinue to full term if the ovaries are removed even before the middle of the gestation period. This is in agreement with results obtained by Loeb and Hesselberg (1917). Mulon (1908) according to Asdell (1928), also working on the guinea pig found that the pregnancy was interrupted when the ovaries were removed at various stages from four to fifty-seven days. He does not state the number of animals used in the experiment.

Loeb (1908) made the unique and interesting discovery that in the guinea pig deciduamatous tumors might be produced by cauterizing the uterus during the early life of the cylic corpora lutea. If the corpora lutea had been removed the formation of deciduamata could not be induced. Corner and Warren (1919) report a similar reaction in the rat if the uterus was cauterized shortly after post partum ovulation. Long and Evans (1922) were unable to produce deciduamata in the uterus of the rat during the cycle but they found that if the life of the corpus luteum was prolonged by stimulation of the cervix by means of a glass rod introduced into the cervical canal and followed by traumitization of the uterus four or five days later excellent deciduamatous tumors might be produced.

Experimental work by Loeb and Hesselberg (1917), and Stockard and Papanicolaou (1920) for the guinea pig, Hammond (1927), for the cow, and others confirm the hypothesis advanced by Beard pertaining to the inhibition of ovulation and oestrous. These workers reported that the removal of the corpora lutea hastened the onset of oestrous and of ovulation.

Experiments pertaining to the relations of the corpus luteum to the mammary glands were probably performed first by Ancel and Bouin (1909). They concluded from their work on the rabbit that the corpus luteum was responsible for the growth changes in the mammary gland during the first half of pregnancy but that some new force was responsible for the further development. Hammond and Marshall (1914) and Hammond (1917) concluded that the development of the mammary gland during the second half of pregnancy was under the same influence as that which controls the early development, and that in both cases the factor is the corpus luteum. These workers found that in pseudo-pregnancy in the rabbit the mammary glands were developed at nineteen days when the corpus has reached the heighth of its development to such a degree of completion as to result in the secretion of milk. Their conclusions were based on 21 experiments. Hysterectomy, they found, had no effect on mammary gland or ovarian development. They concluded that mammary gland development may take place in response to a stimulus from the corpora lutea alone, and that the secretion of milk results whenever the influence causing glandular growth (namely the corpus luteum) is removed or lessened in amount, provided that the development has gone far enough. Loeb (1923) found that in animals where the persistent corpus luteum obtained by hysterectomy lasted for 60 days lactation might be produced.

Other authors believe the continued secretion of oestrin to be responsible for mammary gland development. Herrmann (1915) reported increased growth of the mammary glands of the rabbit following the injection of oestrin. This was confirmed by Ancel and Bouin (1924) for the rabbit and also by Frank and Rosenbloom (1915) for the guinea pig. Allen and Doisy (1923) were able by the injection of the oestrous hormone to induce hypertrophy of the mammary glands. They concluded that this hormone provided the stimulus for mammary development.

Parkes (1928 b) reports that by the injection of a basic extract of the anterior pituitary, he was able to obtain mammary growth in the rabbit in excess of that found during pseudo-pregnancy. This he believes to be caused by the lutealizing effect of the pituitary extract on the ovary.

Experiments endeavoring to obtain the functions attributed to the corpus luteum by means of the injection of extracts of the gland, up to the last few years have been practically without success. Herrmann (1915), one of the earliest workers to experiment in this field, recorded growth in the uterine tract of immature bitches by the injections of a corpus luteum extract.

Later workers have secured more definite reactions. Hisaw, Meyer and Weichert (1928) report relaxation of the symphysis pubis in the guinea pig following the injection of extracts of the corpus luteum. They have been able in addition by the injection of extracts of the corpus luteum to produce other phenomena characteristic of early pregnancy, including sensitization of the uterine mucosa, inhibition of ovulation and alteration of the endometrium of the uterus of the rat.

Papanicolaou (1926), Parkes and Bellerby (1927), Hisaw, Meyer and Weichert (1928), and others have reported inhibition of oestrous and ovulation by the injection of corpus luteum extracts. Methods of extract preparation which were used by these workers differ considerably.

Corner and Allen (1929 a) report the preparation of an alcoholic extract of the corpora lutea of the sow, by means of which they were able to produce progestational proliferation of the uterus in spayed rabbits identical with that of normal pregnancy. Does were ovariectomized about 18 hours after copulation. The animals then received daily subcutaneous injections of the corpus luteum extract in doses from 0.12 cc. to 0.2 cc. until five doses were given. On the fifth day the animals were sacrificed and the genital tract removed for examination. Under the influence of these extracts general growth of the uterus was obtained, the vessels became engorged with blood and characteristic proliferation occurred in the endometrial glands. In a slightly later paper Allen and Corner (1929 b) reported that not only were their extracts potent to produce characteristic proliferation of the uterus in spayed animals but that normal implantation could be produced by means of the extracts. They described two animals which were carried to the thirteenth and seventh days of pregnancy respectively. In a more recent paper, (1930 a), Corner and Allen report successful termination of the pregnancy in animals ovariectomized 18 hours after copulation. Four animals which received from 0.2 cc. to 0.5 cc. daily of an extract known to be potent went to spontaneous delivery on the twenty-eighth, twenty-ninth, thirty-second and thirty-third days respectively. Two animals which received respectively 1.0 cc. and 0.5 cc. of the extract daily throughout the term failed to give birth to their young on the thirty-third day though growth had apparently continued to 26 and 33 days respectively.

Harris and Pfiffner (1929) reported relatively little effect on the duration of the pregnancy in spayed rats, by the use of corpus luteum extracts prepared according to Corner and Allen (1929 a). They reported an average continuation of the pregnancy for almost four days after double ovariectomy. Extirpation of both ovaries from pregnant rats, not followed by injections resulted in the average termination of the pregnancy 2.22 days after the removal of the second ovary. With an extract prepared in the same way, except that methyl alcohol was used instead of ethyl as used by Corner and Allen, these workers report that pregnancy continued to term in eleven of twelve experiments. If only one ovary was removed the administration of this extract interfered with parturition.

PURPOSE

The purpose of this experiment was to investigate the importance of the corpora lutea in the maintenance of pregnancy in the rat by determining the effect of (1) bilateral ovariectomy at various stages of pregnancy and (2) of the injection of extracts of the corpus luteum in animals ovariectomized in the various stages.

MATERIALS AND METHODS

Albino rats in varying stages of pregnancy served as subjects in most of the experiment. Some work was also done on the spermophile.

The stage of pregnancy in all cases where the rat was used was based on the time of copulation or of observing the vaginal plug. Care was taken to make the conditions under which the operations were performed as nearly aseptic as possible and in no case was any infection known to result from the operation. Hair was removed with a 20% solution of sodium sulphide and the region then painted with mercurochrome. Instruments were always sterilized by boiling before using them.

In the early stages only one incision was made in the ventral body wall and both ovaries thus removed. In the later stages two dorso-lateral incisions were made, one directly over each ovary. This was done to prevent handling or moving of the blood gorged uterus. The ovarian artery was tied in each case before removing the ovary. In all cases where positive results were obtained the animal was later sacrificed and examined for any remaining ovarian tissue. Examinations were made with the binocular microscope.

All injections were made subcutaneously. Because of the large fold of loose skin under the shank, injections were made in that region. Animals were injected immediately following the operation and daily thereafter. The usual amount used for one injection was 0.2 cc. to 0.3 cc. In two cases this was increased to 0.4 cc.

For the preparation of the extracts fresh corpora lutea of the sow were obtained from Armour and Company in Kansas City. Healthy looking corpora lutea of all stages of the cycle and of pregnancy, except those in retrogression were used. Estimation of the age was based on the description by Corner's (1921) and only the pink quite solid corpora were used. The corpora were shelled from the rest of the ovary while still warm, ground in a meat grinder and preserved in from 2 to 3 volumes of alcohol. The method of extraction was essentially the same as given by Corner (1929 a).

The procedure in detail for the preparation of the corpus luteum extracts is as follows: The tissue preserved in alcohol was filtered through a bag of cheese cloth and where a kilogram or more of tissue was used, the residue was divided into two equal parts and each part extracted five times with hot alcohol in a Bloor extractor, each extraction lasting for one hour. For the first preparation of extract this technic was changed slightly in that instead of using a Bloor extractor the bag of tissue was put directly into the alcohol and boiled for an hour. The mass of tissue in the bag was kneaded in each case after the second and fourth extractions. About 150 - 250 cc. of alcohol were used for each extraction depending on the exact amount of tissue used. Thus none of the extracted material was heated to the boiling point of alcohol for more than one hour. The alcohol was distilled off by heat under diminished pressure obtained by a good water pump. The temperature very rarely went above 60°C. and usually not above 40°C. When the solvent was almost completely removed the distillation was stopped and the residue extracted five times with peroxide-free ether, about 500 cc. being used for the first extraction and 150 to 200 cc. for each remaining extraction. The ether was redistilled immediately before using and only that distilling over at a temperature of 34.7° C. or lower was used, to guard against the presence of peroxides. The ether solutions were combined and the volume reduced to about 100 cc. or less by vacuum distillation. To this solution four volumes of acetone were added to remove the phospholipins. The precipitate was redissolved in ether and reprecipitated with acetone five times. The acetone-ether solutions were combined and the solvents again removed by vacuum distillation on the steam bath. A heavy brown oil was thus obtained which was removed from the distilling flask to a centrifuge tube with the aid of 50 - 100 cc. of ether. The solution was then centrifuged to remove any ether-insoluble material. The ether layer was pipetted off and the ether remaining in the oily liquid was evaporated off over a warm water bath. A heavy brown semi-liquid oil was thus obtained yielding approximately the same as obtained by Corner, about 20 grams per kilogram of fresh tissue. In the second preparation a larger yield was obtained at first because no separation occurred from centrifuging. The extract was recentrifuged after standing for several days and the usual yield of heavy oil was then obtained.

The extract was placed in sterile bottles with rubber stoppers so that it was not exposed to the air when used.

With the first preparation these precautions were not taken and the extract spoiled because of bacterial invasion. Before injecting, it was necessary to immerse the container in warm water for a short time.

For two of the preparations methyl alcohol was substituted for the ethyl. For this the procedure was exactly as before. The yield in these two cases was slightly lower than with the use of ethyl alcohol. A smaller amount of phospholipin was precipitated on the addition of acetone and the final oily mixture was somewhat more fluid. In the refrigerator where the extracts were kept, that prepared with ethyl alcohol solidified while that prepared with methyl alcohol remained fluid.

Thirty-two animals were used to determine the effect of injections of the extract. Of these, twenty animals were injected with the ethyl alcoholic extract and twelve with the methyl. As controls against these animals (the effect of the extract) a series of twenty experiments was run to determine the effect of double ovariectomy in the various stages without any injections. A series of eight controls from which only one ovary was removed was run against the double ovariectomized animals. The animals remained in good condition except that the extract because of its partly insoluble nature was not entirely absorbed.

TABLE I DOUBLE OVARIECTOMY - NO INJECTIONS

Animal No.	Time of Operation Days	Time Between Operation and Abortion
327	7	36 - 48 hours
107	8	36 - 40 hours
110	9	36 hours
126	9	40 - 48 hours
129	11	36 - 40 hours
102	13	60 - 72 hours
117	14	30 - 36 hours
299	15	36 - 40 hours
133	17	32 - 40 hours
101	18	28 - 36 hours
224	18	40 - 44 hours
217	19	40 hours. All Dead.
203	19	3 days - full term. 3 dead, 4 living. Young all dead - 12 hrs. Mammary glands not functional.
105	19	24 - 36 hrs. All dead.
201	20	36 - 40 hrs. Full term. 7 dead, 3 living, all dead within 12 hours. Mammary glands not functional.
231	20	48 hrs. Full term. 7 dead, 1 living - Dead within 12 hours. Mammary glands not functional.
232	20	48 hrs. Full term. 5 dead, 4 living - All dead within 12 hrs. Mammary glands not functional.
282	20	48 - 52 hrs. Full term. 6 living, 3 dead, only 2 living after 12 hrs. Mammary glands developed.
293	21	40 - 48 hrs. Full term. 8 living young.
278	21	36 - 40 hrs. Full term. 3 dead, 4 living.

RESULTS

The results from twenty experiments of bilateral ovariectomy in stages from seven to twenty-one days of pregnancy, given in Table I, show that the extirpation of both ovaries from pregnant rats always results in the termination of the pregnancy. Results varied some in so far as the duration of the pregnancy was concerned but in every case where ovariectomy was performed previous to nineteen days the pregnancy was interrupted. In all cases in stages from six to eighteen days, except in No. 102, bilateral ovariectomy resulted in the abortion of the foetuses from 28 to 48 hours after the operation. In one case (No. 102) abortion did not occur until from 60 to 70 hours after the operation.

Bilateral ovariectomy at 19 to 20 days resulted in a large proportion of still births and of the young born viable the majority lived less than 24 hours. There was more variation in these stages than in earlier stages. Of the three animals ovariectomized at 19 days, in Nos. 217 and 105 the pregnancy was terminated in less than 48 hours after the operation and so can hardly be considered full term. As shown in Table I there were no viable young in either of these cases. In No. 203, operated at the same stage the pregnancy continued to full term and of seven young, four were viable but died within 12 hours. The mammary glands appeared very small and undeveloped so in order to determine if death was due to the lack of nourishment after birth or to the physical condition of the young, two young rats from a normal litter about one day of age and four rats two days of age were given to No. 203 and No. 232 respectively. In neither case were the young able to suckle and those given to No. 203 died within two days and those to No. 232 within three days. The remaining young from the same litters developed normally. At 20 days viable young were born in each case but in only one case (No. 282) did any of them live longer than 12 hours. Average weights of these young, where weights were taken, ranged from 2.8 to 4.0 grams while the average normal weight at birth was found to be from 4.5 to 5.0 grams. The mammary glands except in No. 282 were apparently not functional so that death of the young may be ascribed to the lack of nourishment.

That these effects were the result of the removal of the ovaries and not a consequence of the operation is shown by a comparison with the results from unilateral ovariectomy given in Table II. The results from eight experiments show that the removal of one ovary has no effect upon the pregnancy independent of the stage.

TABLE II

UNILATERAL OVARIECTOMY

Animal No.	Days of Gestation When Operated	Number of Viable Young
169	10	8
263	11	7
271	12	10
222	13	7
140	14	4
211	19	9
303	20	8
204	21	6

TABLE III DOUBLE OVARIECTOMY FOLLOWED BY INJECTIONS OF ETHYL ALCOHOLIC EXTRACTS OF THE CORPUS LUTEUM

Animal No.	Days of Gestation When Operated	Amount Injected Daily and Days	Effect of Injections and	Degree of Effect
* 259	1 (18 hrs.)	.15 cc. Ex II 9 d.	Proliferation and implantation	+ +
* 264	2	.15 cc. Ex. II 8 d.	Some proliferation	+
* 175	2	.2 cc. Ex. III 8 d.	Some proliferation	+
126	8	.2 cc. Ex. I 2 d.	Bleeding 36 hrs. Abortion 2 d.	-
257	9	.2 cc. Ex. II 7 d.	Bleeding 2 d. Resorption	+
* 263	10	.2 cc. Ex. II 11 d.	Normal development 6 d. Bleeding 7 d. Resorption	+ +
286	12	.2 cc. Ex. II 5 d.	Bleeding 3 d. Resorption	+
109	12	.2 cc. Ex. I 4 d.	Bleeding 2 d. Abortion 4.5 d.	+
119	14	.3 cc. Ex. I 1 d.	Animal Dead 1.5d. Extract Spoiled	
* 252	15	.3 cc. Ex. II 5 d.	Slight bleeding 1 d. Full term still births 5.5 d.	+ +

TABLE III (CONTINUED)

Animal No.	Days of Gestation When Operated	Amount Injected Daily and Days	Effect of Injections and	Degree of Effect
296	16	.2 cc. Ex. III 3 d.	Bleeding 2.5 d. Abortion 3 d.	+
291	17	.25 cc. Ex. III 4 d.	Bleeding 3 d. Abortion 4 d.	+
218	17	.3 cc. Ex. III 3 d.	Bleeding 2 d. Abortion 2.5 d.	-
* 294	18	.3 cc. Ex. III 3 d.	Full term 3 d. 3 dead, 3 viable All died in 12 hrs. Mammary glands not functional	+
* 295	18	.25ccc. Ex. II 3 d.	Full term. 6 living young -1 dead Mammary glands functional	+ +
322	18	.3 cc. Ex. III 2 d.	Abortion 1.5 d.	-
* 281	19	.2 cc. Ex. II 13 d.	Full term. 1 dead, 6 living. Mammary glands functional	+ +
* 272	19	.2 cc. Ex. II 10 d.	Full term. 10 living. Mammary glands functional	+ +
*290	19	.2 cc. Ex II 2 d.	Full term 3 dead, 11 living Mammary glands functional	+ +
241	20.5	.2 cc. Ex II 3 d.	Bleeding 1.5 days, parturition 3 d. 6 dead, none viable	+

Ex. I, II, III refers to the different batches of extract prepared.

* Indicate that examination for remaining ovarian tissue was made and none found.

TABLE IV DOUBLE OVARIECTOMY FOLLOWED BY DAILY INJECTIONS OF METHYL ALCOHOLIC EXTRACT

Animal No.	Days of Gestation When Operated	Amount and Number of Days Injected	Effect from Injections -	Degree of Effect
314	1 (18 hrs.)	.2 cc.;Ex.II; 9 d.	Slight effect on uterus	+
321	1.5	.3 cc.;Ex.II; 6 d.	Slight effect of uterus	+
298	2	.2 cc.;Ex. I; 6 d.	No effect	-
176	4	.2 cc.;Ex. I; 4 d.	No effect	-
315	8	.2 cc.;Ex. I; 5 d.	Bleeding 2 days. Resorption.	+
251	9	.2 cc.; Ex.II;3 d.	Bleeding 1.5 days, abortion 2 days	-
* 337	10	.4 cc; Ex.II; 5 d.	No bleeding. Embryos larger after 2 days. Greatly degenerated at 5 days. Resorption	+ +
223	12	.2 cc;Ex.II; 4 d.	Bleeding 3 days. Abortion 4.5 days	+
* 300	13	.3 cc;Ex. I; 9 d.	Slight bleeding 4 days. Resorption. Gain in weight for 6 days	+ +
330	14	.25cc.;Ex.II;3 d.	Bleeding 1 day, abortion 2 days	-
* 340	14	.2 cc.;Ex.II;7 d.	Bleeding 4 days. Embryos not degenerated at 5 days. Resorption. Embryos badly degenerated at 7 days	+ +
* 336	13	.4 cc;Ex. II; 9 d.	No bleeding. Embryos normal on 23rd day. Partufition apparently inhibited. 5 viable young 24 days. Mammary glands functional.	+ +

Results from 32 experiments of bilateral ovariectomy and the subsequent injection of alcoholic extracts of the corpus luteum as prepared in this experiment vary considerably in the further duration of the pregnancy. Apparently there was a close correlation in the degree of absorption of the injected material and the length of time that the pregnancy was maintained. Animals varied in the degree to which the extract was absorbed. There is some variation in the results obtained from the injection of the methyl and ethyl-alcoholic extract but this was not much greater than the variation between the different extracts prepared in the same way.

For convenience an arbitrary standard as to the degree of effect obtained by means of the extracts was devised. The degrees are designated in Tables III and IV by + and - signs, + + indicating marked effect, + some effect and - no effect.

The results obtained from the injection of extracts prepared with ethyl alcohol in three animals from which the ovaries were removed before implantation show little effect especially when compared with the results obtained by Corner and Allen (1929 a). There was some growth effect on the endometrium of the uterus in each of these animals. This was most marked in No. 259 but apparently was not as great as in normal ten-day pregnancy. It also appeared quite certain that in No. 259 implantation had occurred. Examination of the animal nine days after ovariectomy revealed several enlargements, one in the uterine cavity and four, apparently extra-uterine implantations, in the surrounding fat. Microscopic examination of these bodies indicated that they were masses of degenerating tissue, probably the vestiges of blastocysts. In Nos. 264 and 175 there were no signs of implantation but it is possible that these animals had not conceived, as the finding of a vaginal plug is not an infallible indication that the animal will become pregnant. Quite frequently pregnancy failed to follow after the discovery of a vaginal plug.

In 18 animals operated at later stages as shown in Table III the pregnancy with the exception of three cases, was to external evidence at least, continued normally from two to four days as evidenced by a steady gain in weight and the absence of bleeding from the vagina. In nine animals operated at eight to seventeen days bleeding occurred on the average at 2.75 days. This was followed by abortion in six of the nine animals at an average of 3.6 days after the operation and by the resorption of the foetuses in three animals. In No. 263, operated at ten days, development was apparently normal for six days. Bleeding from the vagina was first noticed on the seventh day after the operation. The animal was anaesthesized and opened at this time to ascertain more definitely the fate of the foetuses. They were found to have made considerable growth and were normal in form so that death had probably not occurred more than one day before. After this bleeding was pronounced and degeneration apparently was rapid.

Of three animals operated at 18 days, in two (Nos. 294 and 295) the pregnancy continued to full term and viable young were born. In No. 294 the mammary glands were not functional and the young died within twelve hours. The average weight of the three viable young was 4.5 grams. Death was apparently due to lack of nourishment. Two rats, 2.5 days old given to No. 294 died within three days. In No. 295 the mammary glands were functional and the young all lived. They seemed weak and averaged only 3.8 grams at birth. Apparently foetal nutrition had not been normal but had been maintained to a sufficient degree to prevent the death of the foetuses. No. 322, operated at the same stage, did not continue to full term, abortion occurring one and one-half days after the operation. As shown in the table, Nos. 294 and 322 received daily injections of extract No. 3 and No. 295 of extract No. 2. It seems possible that the variation in effect may have been the result of a difference in the potency of the different batches of extract.

Three animals operated at 19 days all continued to full term with viable young. In each of these animals the mammary glands were functional. A comparison of these results with those in Table I shows a positive effect of the extract. Of three animals operated at 19 days and not injected only one (No. 203) continued to full term and in this animal the mammary glands were not functional. Four uninjected animals operated at 20 days continued to full term with 36 young only 11 of which were viable and all but two of these died within 12 hours. Since the mammary glands in these animals were not functional while those in the injected group were, development must have been caused by the injections. No. 241 operated at 20.5 days and injected failed to give birth to living young. The extract seemed to interfere with parturition which should have occurred at the time bleeding set in.

The extract is apparently able to continue the functions of the normal corpus luteum for a short time but in the rat a daily dosage from 0.15 to 0.3 cc. is not potent to maintain the normal conditions of pregnancy beyond a few days. Doses of 0.3 cc. appear to have little more effect than smaller amounts.

The extract had no effect on the pregnancy of a normal animal when injected from the nineteenth day on in a dosage of 0.2 cc.; viable young were born on the twenty second day.

Daily injections of 0.4 cc. in two animals that had been coming into oestrous regularly every four to five days inhibited oestrous, as ascertained by vaginal smear examinations. Injections were continued for six and eight days respectively and both animals remained in dioestrum for 10 to 14 days after the last injection.

The results obtained from the extracts prepared with methyl alcohol are given in Table IV. Animals ovariectomized before implantation had occurred and subsequently injected showed less effect on the endometrium of the uterus than those injected with the ethyl alcoholic extracts. Implantation failed to occur in any of four animals.

In the later stages, from eight to 14 days, the effect was in most cases more marked. In two of eight animals (Nos. 315 and 330) the injections had no apparent effect and abortion occurred two days after the operation. In six of eight experiments pregnancy was continued from three to nine days and in only one case did abortion occur. This was at four and one-half days after the ovariectomy. In five of six animals bleeding was followed by resorption of the foetuses. No. 300 operated at 13 days gained in weight for six days but slight bleeding was noticed on the fourth day after the operation. Examination on the ninth day revealed seven greatly degenerated embryos. In No.340 development of the embryos was apparently normal for a period of four days. In No. 337 which received daily injections of 0.4 cc. the embryos had gained in size from 6 x 7 mm. to 10 x 12 mm. 2.5

days after the operation but resorption occurred a day or two later. No vaginal bleeding was observed in this animal. No. 336 which also received daily injections of 0.4 cc. continued to full term nine days after the operation. Parturition seemed to be inhibited but operation on the twenty third day revealed the living foetuses on uteri. Parturition occurred on the twenty fourth day, 2.5 days after the last injection with five viable young. Mammary glands were functional. It seems from this result that a larger dosage may be more effective in continuing the pregnancy than a small one.

Comparison of the results obtained from the methyl and ethyl - alcoholic extracts reveals no great difference in the degree of effect obtained except in No. 336. The most outstanding difference in results is in the greater percentage of resorptions obtained from the methyl extract. Pregnancy was apparently discontinued on an average, at 2.7 days when the ethyl extracts were injected and at 3.7 days after the operation when the methyl extracts were used. Both extracts had a stimulating effect on the mammary glands.

RESULTS FROM SPERMOPHILES

Eight experiments on spermophiles during the spring of 1929 and of 1930 indicate that the extracts are more potent in maintaining the pregnancy in these animals than in rats.

In the spring of 1929 the extract spoiled during the course of the experiment and results were obtained for only one injected animal and its control which was uninjected. Ovariectomy in both animals was performed at an early stage. The embryos were 4 x 4 mm. in size in the experimental animal and 4.5 x 4.5 mm. in the dontrol. Both animals were opened seven days after the operation. In the control the embryos had degenerated into small pus-like masses while in the experimental animal the embryos had grown to 8 x 4.5 mm. The animal was sacrificed five days later and the embryos were then found to have degenerated quite badly.

In the spring of 1930, of seven injected animals ovariectomized at medium to late stages a marked effect on the duration of pregnancy occurred in four cases. The three in which little effect was noted were in poor physical condition when they were brought in from the field so the lack of effect should not be considered as being entirely the result of the ineffectiveness of the extract. One animal continued to full term with five viable young six days after the operation. It is possible that in this animal a very small portion of one ovary was missed when operated. In another animal pregnancy continued normally for nine days, death occurring on the tenth day. Autopsy revealed six foetuses en uteri averaging 35 x 15 mm. in size which is larger than normally at birth. Evidently the extract had interfered with parturition. Both of these animals received daily injections of 0.2 cc. of the extract prepared with methyl alcohol. In two other animals growth of the foetuses had occurred at the time of examination several days after ovariectomy. One of these received daily injections of 0.2 cc. of the methyl extract and the other 0.4 cc. of the ethyl. Double ovariectomy with no injections confirm the results obtained by Dripps (1919), in that gestation was always interrupted.

The results from these experiments suggest a difference in the two species (rat and spermophile) in their reactions to the injections. The extracts are clearly more potent in maintaining pregnancy in the spermophile than in the rat. This variation in effect may possibly be due to a difference in the amounts of the hormone required to maintain pregnancy in the two species since by the injection of 0.4 cc. one rat operated at 13 days continued to full term. However the same amount had little effect on an animal operated at ten days.

DISCUSSION

These results show conclusively that in the rat the corpora lutea are necessary throughout the pregnancy. This is not in accordance with the conclusion obtained by Hammond and Marshall (1914) who considered that the functions of the corpus luteum ceased at about the middle of pregnancy. The results are in accordance with those obtained by recent workers for other animals, as enumerated above, except for the results obtained by Herrick (1928) and Loeb and Hesselberg (1917), for the guinea pig. Harris (1927) found the interval elapsing between the time of removing the ovaries and abortion to average about 22 hours for the mouse. This is in close agreement with the average time found to elapse between ovariectomy and abortion in the rat in this experiment. This was about 36 hours. Parkes (1929 a) conducted an experiment in bilateral ovariectomy on mice in which the follicles of one ovary had previously been obliterated by means of unilateral exposure to X-rays. The mice were later mated and the functional ovary removed at 11 - 17 days of the gestation period. Of 17 animals so treated abortion occurred on an average at two days after the operation.

A comparison of these results obtained by Parkes and Harris for the mouse, with those obtained in this experiment for the rat indicates that it is the corpus luteum that is responsible for the maintenance of pregnancy and that the presence of residual tissue alone has no effect on the pregnancy. This is not in agreement with the idea advanced by Lipschutz (1925) that the interstitial cells may perform the same function as the corpus luteum. The interruption of pregnancy in the cow after squeezing out the corpora lutea as obtained by Hess (1915) and Schmaltz (1917) support the same view.

Results obtained, in this experiment and in others previously described, from the injection of extracts of the corpus luteum in pregnant animals likewise support this conclusion. Allen and Corner (1930) were able by means of corpus luteum extracts to maintain pregnancy in the rabbit to full term when ovariectomy had been performed 18 hours after copulation. In this experiment pregnancy in the rat was maintained in one case for nine days, after the removal of the ovaries, by the injection of a corpus luteum extract. Since the normal conditions of pregnancy may be maintained by the injection of extracts prepared from corpora lutea we may conclude that it is the corpus luteum that normally performs this function.

Experiments by Smith (1926) and by Parkes and Bellerby (1926) on the effect of injection of the oestrous hormone in pregnant animals, resulting in the termination of pregnancy about two days after the first injection, indicate clearly that the oestrous hormone cannot be the main factor in maintaining pregnancy. This does not necessarily indicate that the actions of the two hormones are antagonistic when they occur in their normal proportions. The results obtained by means of corpus luteum extracts in these experiments indicate rather that they have an interrelated effect for no separation of the two hormones was made in the preparation of the extracts. Corner and Allen (1929 a) tested their extract for the presence of oestrin and found that it contained small amounts of this hormone. The theory that the two hormones work together in pregnancy is in agreement with the conclusion reached by Hisaw (1929), that the pregnancy changes in the uterus require the action of both the follicular and corpus luteum hormones.

Termination of pregnancy appears to be caused by two factors, first an interference with the foetal nutrition which is probably the result of a change in the decidua after the removal of the corpora lutea, and second the setting into activity of the mechanism of parturition, that is, the contraction of the walls of the uterus. That this is one of the effects brought about by the withdrawal of the functional corpora lutea is also evidenced by the results obtained from the injections, especially of the methyl-alcoholic extract, which appears to have a pronounced inhibitory effect on the sensitivity of the uterus to the oxytocic hormone of the pituitary. Since the injection of extracts of the corpus luteum has a tendency to bring about absorption rather than abortion it appears that abortion and normal parturition are brought about by the same factor, cessation of the activities of the corpus luteum. This is in agreement with the theory given by Parkes (1928) and first advanced by

Knaus that the corpus luteum when functioning causes the sensitivity of the uterus to pituitrin to decrease and when the corpus luteum atrophies at the end of pregnancy this sensitivity is again increased resulting in the contractions of the uterus and the expulsion of the foetuses. Harris and Pfiffner (1929) state that by the injection of a corpus luteum extract in animals having one ovary removed parturition was inhibited. Similar results were obtained in two animals in this experiment and in two animals by Allen and Corner (1930 a). These results also support this theory as to the cause of parturition.

In five of six animals used in this experiment when by the injection of an extract, pregnancy was continued to full term at three to nine days after ovariectomy, the mammary glands were functional. In four of five injected animals that gave birth to viable young after ovariectomy at 19 - 26.5 days of gestation the mammary glands had not developed to a functional stage. This is not in agreement with the results obtained by Herrick (1927) for the guinea pig. The results in this experiment tend to support the theory advanced by Hammond and Marshall (1914) and confirmed by Parkes (1928 b) that the prime factor concerned in mammary gland development is the corpus luteum. Since by the injection of extracts of the corpus luteum mammary gland development to a functional degree was obtained at stages which in the absence of injections always resulted in non-functioning of the mammary glands, we may conclude that the factor providing the stimulus for this development in the rat is the corpus luteum. If the presence of embryos in the uterus provided the stimulus for mammary gland development the glands should have been functional in the non-injected animals as well as in the injected ones. It seems possible that there is a variation in species as to the stimulus causing the development of the mammary glands. This would account for the difference in results obtained by Herrick for the guinea pig and those obtained in this experiment for the rat. This is not in accordance with the theory of Allen and Doisy (1923) attributing mammary gland development to the follicular hormone. It seems probable however since by the injection of oestrin some mammary growth may be obtained, that both hormones are concerned in the development of the gland.

A comparison of the results obtained by Corner and Allen (1930 a) on the rabbit by the injection of extracts prepared in essentially the same way as in this experiment, suggest the possibility of a difference in species in the reaction to the hormone. That there may be such a difference is further indicated by the difference in results obtained for the rat and the spermophile. When corpus luteum extracts are injected in the same dosage in the spermophile and rat the former clearly shows a more pronounced effect.

The results obtained from the injections of the methyl alcoholic extracts are on the average quite negative as compared with those obtained by Harris and Pfiffner, who report continuation of pregnancy to full term in 11 or 12 animals. It is not stated however at what stage injections were begun or what dosage was used. The results from this experiment show that by the injection of corpus luteum extract the pregnancy in rats may continue to full term in some cases. Results obtained from the injection of the extract prepared with ethyl alcohol are in close accord with those reported by Harris and Pfiffner (1929), the average time to discontinuance being 3.95 days and 3.75 days in this experiment. The results from both of these experiments fail to confirm the results obtained by Corner and Allen (1929 b) and (1930) for the rabbit. The probable explanation for this difference seems to be a variation in the rabbit and rat as to their reactions to the hormone.

CONCLUSIONS

1. The corpus luteum in the rat is functional and necessary throughout pregnancy.

2. By means of the secretion of the corpus luteum the endometrium of the uterus is maintained in a state which makes adequate foetal nutrition possible.

3. The corpus luteum also maintains a hypo-sensitivity of the uterus to the oxytocic hormone of the posterior pituitary thus preventing the contractions of the uterus which result in parturition at the cessation of its activity at the end of the gestation period, and in abortion from its removal during the period of pregnancy.

4. The corpus luteum is essential throughout pregnancy to provide an adequate stimulus for mammary gland development to such a degree that functional activity (the secretion of milk) will result when this stimulus is removed.

5. The reactions characteristic of pregnancy are apparently maintained by an interaction between the corpus luteum and follicular hormones with the corpus luteum in dominance.

6. Double ovariectomy at any time during the gestation period previous to the twentieth day interrupts pregnancy within two days after the operation.

7. When ovariectomy is performed as late as the twentieth day viable young may be born, but the mammary glands are in most cases not functional.

8. The removal of only one ovary has no effect on pregnancy independent of the stage.

9. By the daily injection of 0.2 to 0.3 cc. of an

extract of the corpus luteum prepared with ethyl alcohol the pregnancy may be continued for a period of four or five days after ovariectomy but is in some cases terminated earlier.

10. When ovariectomy is performed after the seventeenth day of gestation and followed by the injection of the extract, the pregnancy continues to full term with viable young and the mammary glands are then usually functional.

11. By the daily injection of 0.4 cc. of an extract of the corpus luteum prepared with methyl alcohol pregnancy in the rat may be continued to full term when the ovaries are removed as early as the thirteenth day of gestation. Smaller amounts usually continue pregnancy for a period of three to four days.

12. The extracts have a more pronounced effect on the duration of pregnancy in the spermophile than in the rat. Apparently there is a difference in the two species in their reactions to the extracts.

13. When pregnancy is continued to full term by the injection of extract the mammary glands are usually functional.

14. The extracts have an inhibitory effect on the sensitivity of the uterus to pituitrin and thus have a tendency to cause resorption instead of abortion and to inhibit parturition.

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